**SUPPLEMENTAL MATERIAL**

## Supplemental Methods

### Patients

Data were collected in a multicenter, observational study design. Eight tertiary pediatric cardiology centers cooperated in the study, as previously described [52]. These eight centers serve almost 100% of the Dutch pediatric population.

Patients (age < 18 years at diagnosis) were included from October 2010 to July 2017. In addition, we retrospectively enrolled children diagnosed with DCM before 2010. The first member of each family who presented to our services with a diagnosis of DCM was designated the proband for this analysis.

DCM was defined as the presence of impaired systolic function (fractional shortening (FS) ≤25%) and left ventricular (LV) dilation (LV end-diastolic dimension (LVEDD) *z*-score >2 for body surface area) [53,54]. Patients with additional structural heart disease that explained their LV dilation were excluded. A diagnostic work-up was performed in all patients, as previously described [11,55]. Patients were subsequently classified into an initial diagnostic category within the six months following their DCM diagnosis. Diagnostic categories consisted of: idiopathic, myocarditis, neuromuscular disease (NMD), familial, IEM, malformation syndrome or other. This classification follows the standard of the Pediatric Cardiomyopathy Registry (PCMR) [11,50]. Familial DCM is defined as two or more affected family members and/or an explanatory genetic finding.

Diagnosis of myocarditis was made based on clinical grounds and viral test results. Myocarditis was 'definite' if there was histological or immune-histological evidence of myocarditis. Myocarditis was 'probable' when blood plasma/serum or cerebrospinal fluid PCR or culture was positive for enterovirus, adenovirus, parechovirus or human parainfluenza virus, or if blood plasma/serum PCR or culture was positive for parvovirus B19, HHV6, cytomegalovirus or Epstein-Barr virus accompanied by serological proof of a primary infection (seroconversion and/or positive IgM) [55]. In the patients

diagnosed before 2010, data on diagnostic work-up, family history and genetic variant segregation analysis were retrospectively collected.

Study endpoint (SE) was defined as all-cause death or heart transplantation (HTx). In addition, patient status at the last follow-up visit was recorded as 'ongoing disease' or 'recovered'. Recovered was defined as two consecutive echocardiograms with normalized LVEDD and FS, with the date of the first normalized echocardiogram considered the date of recovery. Furthermore, gender, age at diagnosis, New York University Pediatric Heart Failure Score (NYUPHFI), NT-proBNP, and standardized echocardiogram (LVEDD, FS) were recorded at inclusion. All study data were collected during routine outpatient clinic visits or hospital admissions. Subjects were followed until SE was reached, the age of 18 years, or the last outpatient visit within the study window. This study was approved by the Medical Research Ethical Committee of the Erasmus University Medical Center (MEC 2014-062) and performed in accordance with the Declaration of Helsinki.

**Genetic evaluation and variant classification**

The genetic data we collected were obtained retrospectively and reflect the genetic evaluation that was common practice at that time: single gene testing (e.g., Sanger sequencing of *MYH7*), targeted next-generation sequencing (NGS) of a gene panel (range 28-70 genes), exome sequencing (ES) with analysis of genes related to cardiomyopathy [7] or open exome analysis. Additional genetic testing (e.g., SNP-array) was performed in a subset of patients in whom a malformation syndrome was suspected. As this was an observational study, patients who had no genetic testing or a test that is now considered too limited were not actively referred for genetic (re)evaluation.

Patients were considered genetically evaluated when at least one genetic test was performed. The pathogenicity of the variants was assessed using Alamut Visual Software (Interactive Biosoftware, Rouen, France). All variants were reclassified (December 2019) by a molecular geneticist specialized in cardiogenetics (RLdD) according to ACMG criteria [56]. Variants with a minor allele frequency <0.1% in the Genome Aggregation Database (gnomAD) were considered rare. Nonsense and frameshift

variants were considered null variants if they occurred proximal to the last 50 bases of the penultimate exon. We defined two groups: patients with a likely pathogenic (LP) or pathogenic (P) variant (class 4 or 5) and patients without a pathogenic variant, including patients with one or more variants of unknown significance (VUS, class 3) [57].

**Statistical analysis**

Categorical variables were reported as numbers and percentages. Continuous variables were reported as means with standard deviation (SD) when normally distributed, or as medians with interquartile range (IQR) when non-normally distributed. To compare clinical characteristics between patients with a LP/P variant and those with negative genetic test results, the student's t-test was used in case of normally distributed variables and the Wilcoxon rank test was used when variables were non-normally distributed. A Chi-square test or two-sided Fisher exact test was performed to examine the relation between categorical data.

We used the Kaplan-Meier method to estimate transplant-free survival in the two groups. The log-rank test was used to determine whether the difference between the two curves was statistically significant. Univariate Cox regression analysis was used to test the predictive value of a LP/P variant. Proportional hazard assumptions were tested, and were not violated. The hazard ratio and 95% confidence interval (CI) were calculated. Testing was performed two-sided, and statistical significance was set at $P < 0.05$. All analyses were performed using IBM SPSS Statistics for Windows, version 24 (IMB Corp, Armonk, NY, USA).

## Supplemental Tables

**Supplemental Table II. Variants of unknown signficance identified in cardiomyopathy-associated genes.**

| Gene | Transcript | Patient | Nucleotide change | Protein change | Classification |
|---|---|---|---|---|---|
| *ALPK3* | NM_020778.4 | P130 | c.5155G>C | p.(Ala1719Pro) | VUS |
| *ANKRD* | NM_014391.2 | P139 | c.642_643delinsT | p.(Arg215Glufs*13) | VUS |
| | NM_014391.2 | P98 | c.651+1G>A | p.(?) | VUS |
| *CAV3* | NM_033337.3 | P66 | c.233C>T | p.(Thr78Met) | VUS |
| *DMD* | NM_004006.2 | P63 | c.1536C>A | p.(His512Gln) | VUS |
| | NM_004006.2 | P96 | c.9955T>C | p.(Cys3319Arg) | VUS |
| *DSC2* | NM_024422.4 | P47 | c.2603C>T | p.(Ser868Phe) | VUS |
| *DSP* | NM_004415.3 | P136 | c.2906C>T | p.(Thr969Ile) | VUS |
| *DTNA* | NM_001390.4 | P117 | c.*5A>G | | VUS |
| | NM_001390.4 | P81 | c.153C>G | p.(His51Gln) | VUS |
| | NM_001390.4 | P81 | c.239G>A | p.(Arg80His) | VUS |
| | NM_001390.4 | P20 | c.784C>T | p.(His262Tyr) | VUS |
| *FLNC* | NM_001458.4 | P143 | c.4413A>T | p.(Gln1471His) | VUS |
| *JUP* | NM_002230.2 | P33 | c.778G>A | p.(Ala260Thr) | VUS |
| | NM_002230.2 | P103 | c.1571T>C | p.(Ile524Thr) | VUS |
| *LAMA4* | NM_001105206.2 | P126 | c.4345C>T | p.(Arg1449Trp) | VUS |
| | NM_001105206.2 | P120 | c.5361_5365del | p.(Lys1787Asnfs*11) | VUS |
| *LDB3* | NM_007078.2 | P81 | c.1051A>G | p.(Thr351Ala) | VUS |
| | NM_007078.2 | P6 | c.2092G>A | p.(Ala698Thr) | VUS |
| | NM_001080114.1 | P37 | c.433G>A | p.(Gly145Ser) | VUS |
| *MYBPC3* | NM_000256.3 | P129 | c.529C>T | p.(Arg177Cys) | VUS |
| | NM_000256.3 | P149 | c.852-20C>A | p.(?) | VUS |
| *MYH6* | NM_002471.3 | P140 | c.4037G>A | p.(Arg1346Gln) | VUS |
| | NM_002471.3 | P81 | c.4193G>A | p.(Arg1398Gln) | VUS |
| *MYH7* | NM_000257.3 | P100 | c.2981A>G | p.Lys994Arg | VUS |
| | NM_000257.3 | P92 | c.495G>A | p.(Met165Ile) | VUS |
| | NM_000257.3 | P18 | c.495G>A | p.(Met165Ile) | VUS |
| | NM_000257.2 | P52 | c.784G>A | p.(Asp262Asn) | VUS |
| | NM_000257.2 | P31 | c.2890G>C | p.(Val964Leu) | VUS |
| *MYOZ2* | NM_016599.4 | P18 | c.148C>T | p.(Leu50Phe) | VUS |
| *MYPN* | NM_032578.3 | P97 | c.59A>G | p.(Tyr20Cys) | VUS |
| | NM_032578.2 | P6 | c.251A>G | p.(Asn84Ser) | VUS |
| *NEXN* | NM_144573.3 | P82 | c.1174C>T | p.(Arg392*) | VUS |
| *PKP2* | NM_004572.3 | P135 | c.184C>A | p.(Gln62Lys) | VUS |
| | NM_004572.3 | P20 | c.1415C>T | p.(Pro472Leu) | VUS |
| | NM_004572.3 | P114 | c.2083C>T | p.(Arg695Cys) | VUS |
| | NM_004572.3 | P135 | c.2258T>C | p.(Leu753Pro) | VUS |
| *PRDM16* | NM_022114.3 | P79 | c.1882G>A | p.(Asp628Asn) | VUS |
| *RAF1* | NM_002880.3 | P140 | c.29C>T | p.(Thr10Met) | VUS |
| *RBM20* | NM_001134363.2 | P12 | c.2381G>A | p.(Arg794Lys) | VUS |
| | NM_001134363.2 | P37 | c.2381G>A | p.(Arg794Lys) | VUS |
| | NM_001134363.2 | P52 | c.2761A>T | p.(Ile921Phe) | VUS |

| | | | | | |
|---|---|---|---|---|---|
| *SCN5A* | NM_198056.2 | P81 | c.2512G>A | p.(Ala838Thr) | VUS |
| *TLL1* | NM_012464.4 | P31 | c.2087A>C | p.(Glu696Ala) | VUS |
| *TNNC1* | NM_003280.2 | P146 | c.47A>G | p.(Gln16Arg) | VUS |
| | NM_003280.2 | P114 | c.202G>A | p.(Gly68Ser) | VUS |
| | NM_003280.2 | P101 | c.205A>G | p.(Ser69Gly) | VUS |
| | NM_003280.2 | P81 | c.304C>T | p.(Arg102Cys) | VUS |
| *TTN* | NM_133378.4 | P1 | c.8707T>C | p.(Cys2903Arg) | VUS |
| | NM_133378.4 | P24 | c.76645G>A | p.(Val25549Ile) | VUS |
| | NM_001267550.2 | P103 | c.13048G>A | p.(Val4350Met) | VUS |
| | NM_001267550.2 | P48 | c.16576A>C | p.(Asn5526His) | VUS |
| | NM_001267550.2 | P91 | c.34283C>T | p.(Pro11428Leu) | VUS |
| | NM_001267550.2 | P33 | c.35189_35191del | p.(Glu11730del) | VUS |
| | NM_001267550.2 | P138 | c.46054G>A | p.(Asp15352Asn) | VUS |
| | NM_001267550.2 | P116 | c.56083G>A | p.(Glu18695Lys) | VUS |
| | NM_001267550.2 | P113 | c.59189C>T | p.(Ala19730Val) | VUS |
| | NM_001267550.2 | P104 | c.66820C>T | p.(Arg22274Cys) | VUS |
| | NM_001267550.2 | P113 | c.73994C>T | p.(Thr24665Met) | VUS |
| | NM_003319.4 | P64 | c.20047A>C | p.(Asn6683His) | VUS |
| | NM_003319.4 | P64 | c.43331T>G | p.(Val14444Gly) | VUS |
| | NM_003319.4 | P143 | c.8604G>T | p.(Trp2868Cys) | VUS |
| | NM_001267550.1 | P95 | c.43700G>C | p.(Arg14567Thr) | VUS |
| | NM_001267550.1 | P95 | c.55355C>G | p.(Ser18452Cys) | VUS |
| | NM_133378.4 | P88 | c.15125T>C | p.( | VUS |
| | NM_133378.4 | P69 | c.60842A>T | p.(Asp20281Val) | VUS |
| | NM_133378.4 | P88 | c.80887C>T | p.(Leu26963Phe) | VUS |
| | NM_133378.4 | P47 | c.80981G>A | p.(Gly26994Asp) | VUS |
| | NM_133378.4 | P88 | c.81029G>A | p.(Arg27010His) | VUS |
| | NM_133378.4 | P88 | c.89826T>A | p.(Asp29942Glu) | VUS |

**Supplemental Table III. Number of likely pathogenic or pathogenic variants normalized to frequency of analysis**

| Gene | Total | Number of LP/P variants | Percentage |
|---|---|---|---|
| ***MYH7*** | 92 | 9 | 9,8 |
| ***MYBPC3*** | 90 | 1 | 1,1 |
| ***LMNA*** | 89 | 1 | 1,1 |
| ***TNNT2*** | 89 | 2 | 2,2 |
| ***TPM1*** | 88 | 3 | 3,4 |
| ***TCAP*** | 87 | | 0,0 |

| | | | |
|---|---|---|---|
| ***TNNI3*** | 87 | | 0,0 |
| ***CSRP3*** | 85 | | 0,0 |
| ***DES*** | 85 | 1 | 1,2 |
| ***EMD*** | 85 | | 0,0 |
| ***LAMP2*** | 85 | | 0,0 |
| ***LDB3*** | 85 | | 0,0 |
| ***TAZ*** | 85 | 1 | 1,2 |
| ***ACTC1*** | 84 | | 0,0 |
| ***GLA*** | 84 | | 0,0 |
| ***MYL2*** | 84 | 1 | 1,2 |
| ***PLN*** | 84 | 1 | 1,2 |
| *TTN* | 84 | 3 | 3,6 |
| ***MYL3*** | 83 | | 0,0 |
| ***TNNC1*** | 83 | | 0,0 |
| ***VCL*** | 83 | | 0,0 |
| ***BAG3*** | 82 | | 0,0 |
| ***CAV3*** | 82 | | 0,0 |
| ***CRYAB*** | 82 | | 0,0 |
| ***PRKAG2*** | 82 | | 0,0 |
| ***JPH2*** | 80 | | 0,0 |
| ***MYPN*** | 80 | | 0,0 |
| ***RBM20*** | 80 | | 0,0 |
| ***SCN5A*** | 80 | 1 | 1,3 |
| ***ACTN2*** | 79 | | 0,0 |
| ***ANKRD1*** | 79 | | 0,0 |
| ***LAMA4*** | 79 | | 0,0 |
| ***NEXN*** | 79 | | 0,0 |
| ***CALR3*** | 78 | | 0,0 |
| ***FHL1*** | 78 | | 0,0 |
| ***MYOZ2*** | 77 | | 0,0 |
| ***TTR*** | 76 | | 0,0 |
| ***DSC2*** | 75 | | 0,0 |
| ***DSG2*** | 75 | | 0,0 |
| ***DSP*** | 75 | 1 | 1,3 |
| ***JUP*** | 75 | | 0,0 |
| ***PKP2*** | 75 | | 0,0 |
| *ABCC9* | 75 | | 0,0 |
| ***MYH6*** | 73 | | 0,0 |
| ***CTNNA3*** | 70 | | 0,0 |
| ***MIB1*** | 70 | | 0,0 |
| ***TMEM43*** | 68 | | 0,0 |
| *SGCD* | 66 | | 0,0 |

| | | | |
|---|---|---|---|
| *FKTN* | 62 | | 0,0 |
| *RYR2* | 61 | 2 | 3,3 |
| *DMD* | 60 | 5 | 8,3 |
| *DTNA* | 59 | | 0,0 |
| *ILK* | 59 | | 0,0 |
| *MYOZ1* | 58 | | 0,0 |
| *PRDM16* | 57 | | 0,0 |
| *MYLK2* | 55 | | 0,0 |
| *CASQ2* | 53 | | 0,0 |
| *FLNC* | 53 | | 0,0 |
| *EYA4* | 51 | | 0,0 |
| *GATAD1* | 51 | | 0,0 |
| *PDLIM3* | 51 | | 0,0 |
| *TBX20* | 51 | 1 | 2,0 |
| *TGFB3* | 48 | | 0,0 |
| *NEBL* | 46 | | 0,0 |
| *TXNRD2* | 45 | | 0,0 |
| *ANO5* | 44 | | 0,0 |
| *HCN4* | 44 | | 0,0 |
| *TMPO* | 44 | | 0,0 |
| *ACAD9* | 36 | | 0,0 |
| *ALPK3* | 36 | | 0,0 |
| *NKX2-5* | 36 | 1 | 2,8 |
| *RAF1* | 35 | | 0,0 |
| *TRIM63* | 34 | | 0,0 |
| *ACADVL* | 31 | | 0,0 |
| *FKRP* | 31 | | 0,0 |
| *ACTA1* | 30 | | 0,0 |
| *AGL* | 30 | | 0,0 |
| *GAA* | 30 | | 0,0 |
| *PLEC* | 30 | | 0,0 |
| *PNPLA2* | 30 | | 0,0 |
| *SOD2* | 30 | | 0,0 |
| *AARS2* | 29 | | 0,0 |
| *AGK* | 29 | | 0,0 |
| *ALMS1* | 29 | 2 | 6,9 |
| *CPT2* | 29 | | 0,0 |
| *DNAJC19* | 29 | | 0,0 |
| *KCNQ1* | 29 | 1 | 3,4 |
| *NDUFA1* | 29 | | 0,0 |
| *NDUFA11* | 29 | | 0,0 |
| *NDUFAF2* | 29 | | 0,0 |

| | | | |
|---|---|---|---|
| *NDUFS1* | 29 | | 0,0 |
| *NDUFS2* | 29 | | 0,0 |
| *NDUFS3* | 29 | | 0,0 |
| *NDUFS4* | 29 | | 0,0 |
| *NDUFV1* | 29 | | 0,0 |
| *NUBPL* | 29 | | 0,0 |
| *PCCA* | 29 | 1 | 3,4 |
| *PSEN1* | 29 | | 0,0 |
| *PSEN2* | 29 | | 0,0 |
| *SCO2* | 29 | | 0,0 |
| *SDHA* | 29 | | 0,0 |
| *SDHAF1* | 29 | | 0,0 |
| *SGCA* | 29 | | 0,0 |
| *SYNE1* | 29 | | 0,0 |
| *TMEM70* | 29 | | 0,0 |
| *ARSB* | 28 | | 0,0 |
| *ATPAF2* | 28 | | 0,0 |
| *BRAF* | 28 | | 0,0 |
| *COA5* | 28 | | 0,0 |
| *COX10* | 28 | | 0,0 |
| *COX14* | 28 | | 0,0 |
| *DOLK* | 28 | | 0,0 |
| *EPG5* | 28 | | 0,0 |
| *FAH* | 28 | | 0,0 |
| *FHL2* | 28 | | 0,0 |
| *GLB1* | 28 | 1 | 3,6 |
| *HADHA* | 28 | | 0,0 |
| *HRAS* | 28 | | 0,0 |
| *IDUA* | 28 | | 0,0 |
| *KRAS* | 28 | | 0,0 |
| *MAP2K1* | 28 | | 0,0 |
| *MAP2K2* | 28 | | 0,0 |
| *MUT* | 28 | | 0,0 |
| *NDUFAF1* | 28 | | 0,0 |
| *NDUFAF3* | 28 | | 0,0 |
| *NDUFAF4* | 28 | | 0,0 |
| *NDUFAF5* | 28 | | 0,0 |
| *NDUFB3* | 28 | | 0,0 |
| *NDUFS6* | 28 | | 0,0 |
| *NDUFV2* | 28 | | 0,0 |
| *NF1* | 28 | | 0,0 |
| *NRAS* | 28 | | 0,0 |

| | | | |
|---|---|---|---|
| *PCCB* | 28 | | 0,0 |
| *PTPN11* | 28 | | 0,0 |
| *RIT1* | 28 | | 0,0 |
| *SHOC2* | 28 | | 0,0 |
| *SLC22A5* | 28 | | 0,0 |
| *SLC25A20* | 28 | | 0,0 |
| *SOS1* | 28 | | 0,0 |
| *SPEG* | 28 | 1 | 3,6 |
| *TNNI3K* | 28 | | 0,0 |
| *TSFM* | 28 | | 0,0 |
| *COX20* | 27 | | 0,0 |
| *COX6B1* | 27 | | 0,0 |
| *FXN* | 27 | | 0,0 |
| *HADHB* | 27 | | 0,0 |
| *HFE* | 27 | | 0,0 |
| *IDH2* | 27 | | 0,0 |
| *MLYCD* | 27 | | 0,0 |
| *SLC25A4* | 27 | | 0,0 |
| *CHRM2* | 26 | | 0,0 |
| *FOXD4* | 26 | | 0,0 |
| *HSPB7* | 26 | | 0,0 |
| *MRPL44* | 26 | | 0,0 |
| *MURC* | 26 | | 0,0 |
| *SGCG* | 20 | | 0,0 |
| *N2A* | 19 | | 0,0 |
| *Novex-1* | 19 | | 0,0 |
| *Novex-2* | 19 | | 0,0 |
| *Novex-3* | 19 | | 0,0 |
| *LAMA2* | 18 | | 0,0 |
| *NEB* | 17 | | 0,0 |
| *SGCB* | 17 | | 0,0 |
| *APOA1* | 15 | | 0,0 |
| *FLNA* | 15 | | 0,0 |
| *ASNA1* | 14 | 1 | 7,1 |
| *CTF1* | 14 | | 0,0 |
| *FLT1* | 14 | | 0,0 |
| *HOPX* | 14 | | 0,0 |
| *NFKB1* | 14 | | 0,0 |
| *SYNM* | 14 | | 0,0 |
| *TRIM54* | 14 | | 0,0 |
| *TRIM55* | 14 | | 0,0 |

## Supplemental Figures

## Supplemental Figure I. Genes versus the number of times it was tested in patients